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ORIGINAL ARTICLE

Intravesical oxybutynin protects the vesical wall against functional and smooth muscle changes in rabbits with detrusor overactivity

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Abstract
Introduction and hypothesis The aim of this study was to evaluate the protective effect of intravesical oxybutynin on the bladder wall of rabbits with detrusor overactivity and partial bladder outlet obstruction (PBOO).
Methods Forty-five North Folk male rabbits were randomly distributed into GI, used as control (n=15), GII-PBOO (n=14), and GIII-PBOO + intravesical oxybutynin (n=15). Connective tissue and elastic fibers were quantified as volumetric density on picrosirius red and Weigert's Fuchsin-Resorcin-stained sections, respectively.
Results In T2, bladder weight was significantly higher in animals in GII and GIII. Smooth muscle bundle diameter was increased by 42% in GII compared with GI (p<0.02). Elastic fibers were significantly higher in GII and GIII as compared with control group. Collagen concentration in GIII and GII was significantly lower than G1 (p<0.025).
Conclusion Intravesical oxybutynin protected against structural and functional detrusor modifications of the partially obstructed bladder.

Keywords Rabbits · Collagen · Elastic tissue · Oxybutynin · Overactive bladder

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Abbreviations

DO	Detrusor overactivity
PBOO	Partial bladder outlet obstruction
GI	Group 1
GII	Group 2
GIII	Group 3
OAB	Overactive bladder
T0	Initial moment
T1	After 1 week of surgical procedure
T2	After 5 weeks of surgical procedure

Introduction

Overactive bladder (OAB) can be caused by several factors such as partial bladder obstruction, aging, or neurological diseases. It is clinically characterized by symptoms such as frequency, nocturia, and urgency, with or without urinary incontinence, and may have important psychosocial implications, including repercussions on the upper urinary tract [1]. In addition, urodynamic studies often indicate detrusor overactivity (DO) in cases of benign prostatic hyperplasia with OAB symptoms [2], as estimated in more than 50% of men with partial bladder outlet obstruction (PBOO).

The mechanical properties of the bladder wall depend on the resistance, viscosity, and elasticity of the detrusor muscle and connective tissue. Elastic system fibers and collagen are major components of this connective tissue and are related to vesical compliance [3, 4]. An abnormal increase in the amount of collagen and elastic fibers commonly occurs in most congenital or acquired obstructive uropathies, possibly leading to a loss of strength and elasticity of the bladder wall [4, 5]. The result of this fibrotic reaction is low compliance, reduced bladder

capacity, and high storage pressure, which may be related to the occurrence of DO [2, 6].

The clean intermittent catheterization associated with intravesical oxybutynin has been used efficiently for the treatment of DO. This efficiency is noticeable especially in patients with significant side effects from the use of oral anticholinergic and already requiring intermittent catheterization, as in those suffering from neurogenic bladder and spinal cord injury, or in children with myelomeningocele [7]. Oxybutynin effects include blockade of muscarinic receptors in the bladder, direct relaxation of the detrusor muscle, and local anesthesia, thereby reducing the frequency of involuntary detrusor contractions and thus improving the OAB symptoms [8]. Further, intravesical administration of oxybutynin can decrease its adverse effects, hence improving its clinical effectiveness.

Morphological alterations in the vesical wall occur frequently in PBOO and include progressive hypertrophy of smooth muscle and changes in collagen and fibers of the elastic system [3, 9, 10]. Additionally, we have shown recently that PBOO is associated with ultrastructural cellular damage in the bladder, which can be prevented by intravesical oxybutynin [11]. However, little is known about the changes in the composition of vesical connective tissue and smooth muscle that underlie the beneficial effects of oxybutynin on DO [12].

This study aims to evaluate the effects of intravesical oxybutynin on major components of bladder wall, such as smooth muscle, collagen, and elastic fibers, using morphological and biochemical methods and an experimental rabbit model of DO.

Materials and methods

This study was approved by the Ethics Committee on Animal Experimentation of the Universidade Estadual Paulista, Botucatu, São Paulo, Brazil.

Animals and treatments

Forty-five North Folk male rabbits, weighting on average 2,207.4 g (range, 1,700 to 3,200 g), were randomly divided into three groups. Group I (GI, n=15) was not submitted to surgical interventions or treatments and was used as control. The experiments started at T0 when the remaining animals underwent PBOO as previously described [13]. Briefly, an adjustable polyethylene bracelet was passed around the bladder neck and was then adjusted so as to not constrict the urethra, which had been previously catheterized with a Foley 10F catheter. This technique produces a mild PBOO, which is not accompanied by significant urodynamic changes, but it is capable of inducing DO [13]. Twenty-nine animals developed DO, as verified by a cystometric study conducted 1 week after surgery (T1). These animals were then randomly divided into groups II (GII, n=14) and III (GIII, n=15). In GIII, the animals were treated with an intravesical instillation of liquid oxybutynin (0.5 mg/kg body weight, daily for 4 weeks). For this treatment, the bladder was first emptied with a 10-Fr catheter, which was promptly removed after drug instillation. The oxybutynin remained in the bladder until spontaneous emptying. Surgical procedures were carried out under intramuscular ketamine and xylazine general anesthesia.

Urine culture, serum creatinine, and cystometric evaluation were performed on all animals at T1 and 5 weeks (T2) after surgery. Animals with positive urine culture at T1 were treated with 1 mg/kg/day of intramuscular trimethropim (Fig. 1).

All animals were killed at T2, and their bladders were removed and immediately weighed immediately using a precision digital scale. The bladders were then fixed in a 10% buffered formalin solution for 48 to 72 h.

Cystometric evaluation

This was performed with in awake animals immobilized in wooden cages and using the Urobyte™ 5000 (Promedon, Buenos Aires, Argentina) computerized urodynamics system as previously published [12, 13]. The intravesical pressure was measured with a 10-Fr double lumen catheter, and the intra-abdominal pressure was assessed by means of

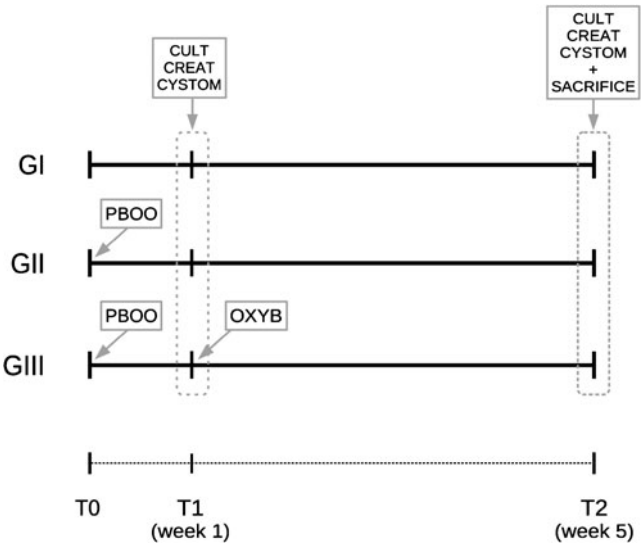

Fig. 1 Diagramatic representation of the experimental protocol. The experiment started at T0, when animals in groups GII and GIII underwent partial bladder outlet obstruction (*PBOO*). Controls (*GI*) consisted of intact animals. One week later (*T1*), oxybutynin (*OXYB*) daily treatment was started in GIII and lasted 4 weeks. At the end of this period (*T2*), all animals were killed. At T1 and T2, urine culture (*CULT*), serum creatinine assay (*CREAT*), and cystometry (*CYSTOM*) were carried out in all groups

a rectal balloon catheter. The maximum bladder capacity, maximum detrusor pressures, and bladder compliance were determined for each rabbit. DO was considered when involuntary detrusor contractions with low vesical volume (<10 mL) were observed, causing or not simultaneous urinary loss. At the beginning of the experiment, urine specimens were collected in sterile tubes for culture.

Histology and morphological quantification

Formalin-fixed tissue samples were routinely processed for paraffin embedding and cut into 5-μm sections showing the full thickness of the bladder wall. Identification and stereological quantification of smooth muscle, collagen, and elastic system were done by staining these sections with hematoxylin and eosin (HE), Picrosirius red [14], and Weigert's Resorcinol-Fuchsin with prior oxone 10% oxidation [15], respectively.

HE-stained images were captured at a magnification of ×250. The largest thickness (μm) of transversely sectioned muscle bundles was measured on five random fields using the Image-Pro Plus (Version 6.3, Media Cybernetics, Bethesda, USA) morphometry program.

For the morphological quantification of connective tissue, images were taken at ×100, and four random fields were analyzed for each animal. In these images, vesical wall tissue fully occupied each field, and the area of connective tissue was measured as μm^2.

The quantification of elastic fibers was done on images taken at a ×1,000 magnification. After sectioning three different areas of the bladder, three random fields in the lamina propria and three in the muscle layer were analyzed, totaling 18 fields per animal. The ImageJ version 1.4 software program (NIH, Bethesda, USA) was then used to generate a 100-point grid, which was juxtaposed to the digital images. The area density of the elastic fibers was quantified separately in the lamina propria and in the muscular layer by point counting and expressed as a percentage of the reference space, as described [16].

All images were digitally captured at a resolution of 1,280×1,024 pixels using a video camera (Leica Microsystems Ltda,Type DFC 280, Heerbrugg, Germany) coupled to a light microscope (Leica DMLB, Leica Mikroskopie & Systeme Gmbh Wetzlar Type 020-518.500 MD/LS, Wetzlar, Germany) and to a computer.

Biochemical determination of the collagen concentration

Bladder tissue samples were fixed in cold acetone immediately after excision and were kept in this fixative for 24 h at 4°C. The samples were then finely minced and submitted to two changes 24 h each in 40 mL of chloroform–methanol solution (2:1, *v*/*v*), at room temperature. The solvent was then decanted, and after incubation at 60°C for 30 min, a preparation of dry and defatted vesical tissue was obtained and weighed. The concentration of total collagen was determined on this preparation by a colorimetric hydroxyproline assay. Thus, 10 to 20 mg of dry bladder tissue was hydrolyzed in 6 N HCl 6 N for 18 h at 118°C as previously described [17]. The assay was then carried out in the neutralized hydrolyzates using a chloramin T method [18]. Results were expressed as micrograms of hydroxyproline per milligram of dry, defatted tissue.

Statistical analysis

The study of the urine culture combination and also of the non-inhibited contractions in the different groups, at the beginning and end of the evaluation, was performed with the MacNemar test. The one-factor analysis of variance (ANOVA) mode was used, together with the Tukey test for multiple comparisons between all pairs of averages. The Pearson correlation coefficient was used for the association between pairs of variables. The one-way ANOVA was also used to compare measures between independent groups, in the assessment of hydroxyproline, as well as Bonferroni's test for planned pairwise comparisons [19]. Differences were considered significant for $p<0.05$ value.

Results

The body weight of animals was increased significantly in all groups at T2, and at this time point, there was no significant difference between groups. Thus, the surgical and pharmacological interventions did not affect the normal growth of animals. Likewise, serum creatinine remained within normal levels, without any significant changes after the experimental procedure.

At T1, urinary tract infection was not found in any animal in GI, but was present in 80% of group GII and in 40% of GIII. At T2, 13% of the GI animals had urinary tract infection, 93% in GII, and 87% in GIII. Urinary infection increased significantly by 47% in group GIII ($p<0.05$).

At T2, in the obstructed animals (GIII) which received intravesical oxybutynin, a significantly lower bladder weight was noted when compared with those that did not receive the drug (GII) (6.00±2.18 vs. 9.61±5.36, $p<0.05$). PBOO (groups II and III) caused a significant increase in bladder weight when compared with the non-obstructed animals (GI) (9.61±5.36 and 6.00±2.18 vs. 2.35±0.55, respectively, $p<0.05$).

In group GII (with partial obstruction), the maximum bladder capacity was significantly higher at T2 in comparison T1 (54.53±22.77 mL vs. 35.13±13.60 mL, $p<0.05$).

There was no statistically significant difference between the groups at different times (Table 1).

For the maximum detrusor pressure, there was no statistically significant difference between groups at T1 and T2.

The bladder compliance was significantly higher in animals with partial bladder outlet obstruction treated with intravesical oxybutynin (GIII) at T2, when compared with T1 (7.19±4.20 vs. 4.67±3.33 mL/cm H_2O). There was no statistical difference between the GI and GII groups at different times (Table 2).

At T2, DO was detected in all animals of GII, in 47% of GIII, and in none of GI. GII animals presented significantly more DO as compared with GI and GIII ($p<0.05$). DO was significantly reduced by 57% in GIII when compared T2 and T1.

Smooth muscle bundles were significantly thicker in the obstructed group (GII) with regards to GI and GIII (180.2± 33.2 μm vs. 126.8 μm±27.3 and 133.1 μm±41.2, $p<0.05$). There was no statistically significant difference between the GI and GIII groups. So, there was an increase of 42.1% in muscle bundle thickness in obstructed group, indicating a protective action of oxybutynin.

The amount of connective tissue was significantly higher in the control group animals with relation to the groups GII and GIII (461,807.1±118,695.3 μm^2 vs. 269,637.9 μm^2± 147,046.2 and 292,879.7 μm^2±106,548.9, $p<0.05$). There was no statistically significant difference between the GII and GIII groups.

The concentration of total collagen was affected in these experimental conditions, although values of the GIII group were not different from those of GII (48.00±11.16 vs. 49.56±13.63, respectively). Thus, these two groups were compared as a set against GI (59.93±11.84), and the results showed that bladder outlet obstruction caused a decrease of about 18% in vesical collagen content (F test, $p<0.025$) (Fig. 2), which was not prevented by oxybutynin.

The percentage of the elastic system fibers, whether in the lamina propria or in the muscle layer, was significantly lower in the GI group compared with GII and GIII (Table 3). There was no statistical difference between GII and GIII.

In the linear association analysis between variables, we observed a negative correlation between the collagen concentration when compared with the percentage of elastic fiber, whether in the lamina propria or the muscle layer and also in muscle fiber thickness (the correlation coefficient= −0.349, −0.457, and −0.417, respectively, $p<0.05$).

There was a positive correlation between the percentage of elastic system fibers of the lamina propria and the muscle layer (the correlation coefficient=0.849, $p<0.05$).

Table 1 Maximum cystometric capacity (mL) in rabbits submitted to no intervention (GI), to bladder obstruction only (GII), and to obstruction followed by oxybutynin treatment (GIII)

Group	Time point		Paired t test
	T1	T2	
GI	36.13±24.78	42.33±21.01	$p>0.05$
GII	35.13±13.60	54.53±22.77	$p<0.05$
GIII	49.87±17.02	51.40±22.73	$p>0.05$
ANOVA	$p>0.05$	$p>0.05$	

Capacities were measured 1 (T1) and 5 weeks (T2) after surgical bladder obstruction. Values are mean ± SD from 15 (GI), 14 (GII), and 15 animals (GIII)

Table 2 Vesical compliance (mL/cm H_2O) in rabbits submitted to no intervention (GI), to bladder obstruction only (GII), and to obstruction followed by oxybutynin treatment (GIII)

Group	Time point		Paired t test
	T1	T2	
GI	2.79±1.47	3.40±1.71	$p>0.05$
GII	2.81±2.10	2.83±1.67	$p>0.05$
GIII	4.67±3.33	7.19±4.20	$p<0.05$
ANOVA	$p>0.05$	$p>0.05$	

Compliances were measured 1 (T1) and 5 weeks (T2) after surgical bladder obstruction. Values are mean ± SD from 15 (GI), 14 (GII), and 15 animals (GIII)

Discussion

The onset and progression of DO in a patient, which is second to a partial bladder obstruction or neuropathy, can cause gradual loss of bladder compliance, hence reducing the capacity and increasing the storage bladder pressure [4–6]. Accordingly, the treatment for obstruction or for detrusor

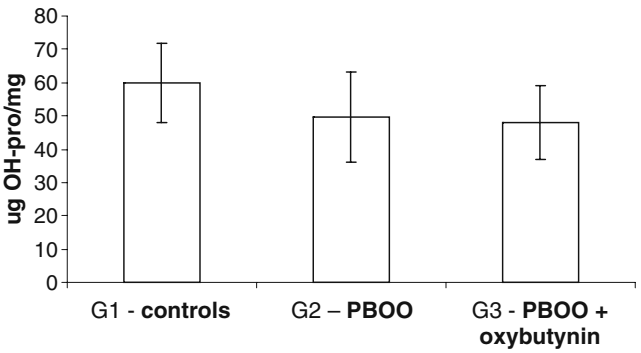

Fig. 2 Concentration of total collagen in the bladder wall of rabbits submitted to no intervention (GI), bladder obstruction only (GII), and obstruction followed by oxybutynin treatment (GIII). Collagen concentration was determined biochemically and expressed as micrograms of hydroxyproline (OH-pro) per milligram of dry vesical tissue. The *bars* represent mean and SD

Table 3 Relative content (%) of elastic fibers (ELAST) and linear thickness (μm) of muscle bundles (MUSC) in the bladder wall of rabbits submitted to no intervention (GI), to bladder obstruction only (GII), and to obstruction followed by oxybutynin treatment (GIII)

Morphometric parameter	Group			ANOVA
	GI	GII	GIII	
ELAST-LP	15.3±3.4	26.2±6.1[a]	24.5±5.1[a]	$p<0.05$
ELAST-ML	13.9±3.3	27.9±6.4[a]	28.2±4.5[a]	$p<0.05$
MUSC	126.9±27.3	180.3±33.2[b]	133.2±41.2	$p<0.005$

Elastic fiber content was determined as area density separately in the lamina propria (LP) and muscle layer (ML). Values are mean ± SD from 13 (GI), 14 (GII), and 15 animals (GIII)

[a] Significantly different from GI at $p<0.05$ using the Bonferroni test

[b] Significantly different from GI at $p<0.02$ using the Bonferroni test

overactivity should occur as early as possible, before progressing to a bladder fibrosis process.

Our results showed that after 4 weeks, the bladder weight was significantly higher in the animals of groups GII and GIII with regards to GI. However, we observed a significantly lower weight in the group treated with intravesical oxybutynin (GIII) compared with the untreated group (GII), thus demonstrating that intravesical oxybutynin has a possible protective effect, in view of the fact that involuntary detrusor contraction (IDC) also disappeared in 53% of the animals in GIII. Increased weight of the bladder has also been described as a frequent change in other studies on partial bladder obstruction and may be related to an increased bladder wall thickness due to edema, depending on the length of time of obstruction, and also to changes in the connective tissue and muscle layer, due to hypertrophy and hyperplasia [9, 12].

In our work, serum creatinine was within normal values in the different groups and moments. This fact shows that renal function was not compromised due to the surgical procedures or due to morphological and functional changes of the bladder.

Significant urinary infection increase in group GIII suggests that PBOO and manipulation of the catheter to apply intravesical oxybutynin are potential risk factors for urinary tract infection. All animals were treated with intramuscular trimethropim to avoid the interference of this factor in the functional and structural analysis of the bladder. Nevertheless, a previous study has shown that the change in urinary pH and the presence of infection do not influence the absorption of oxybutynin [20].

The cystometric study showed a significant increase of the maximum bladder capacity in group GII, when comparing T1 and T2, probably due to the obstruction process, as also demonstrated by Matsumoto et al. [21], after 3 weeks of partial bladder obstruction.

In the present study, no significant change in the maximum detrusor pressure was found between the different times points in the groups. As for bladder compliance, it was significantly higher in animals subjected to intravesical oxybutynin (GIII) at T2 when compared with T1, due to the protective action of this drug [7].

After 4 weeks of treatment with intravesical oxybutynin (T2), involuntary detrusor contractions disappeared in 53% of the animals in GIII, demonstrating the effectiveness of intravesical oxybutynin. At T2, all animals retained IDC in GII, thus suggesting that this is a good experimental model of DO.

In the histomorphometric assessment, muscle bundle thickness increased by approximately 42%, comparing animals of GII with GI. This finding is in line with the information reported by other studies [9, 12]. In GIII, the thickness of the muscle bundle was not different from that of the control group, suggesting a good response to the treatment with oxybutynin. This protective effect is probably due to the decrease of DO, as shown herein. Protection of smooth muscle can also be explained by the fact that oxybutynin improves cellular respiratory function [11].

Studies by Uvelius and Mattiasson [9] and by Kim et al. [22] showed that, in rats with PBOO, collagen concentration in the bladder wall was decreased. Such observation is in agreement with the present study, which also demonstrated a decreased collagen concentration in bladders from groups GII and GIII, both in the biochemical and histomorphometric assessments. Despite of a reduction in DO (53%) in group GIII, these animals were still obstructed, and this fact might explain the absence of improvement in the collagen content in this group.

Evaluating the bladder of patients with PBOO, Cortivo et al. [4] observed an increase in elastic fiber. A similar finding was reported for patients with advanced PBOO due to benign prostatic hyperplasia [5, 10, 23]. Thus, these studies are consistent with our results, which showed an increase of the elastic fiber in the lamina propria and muscle layer in groups GII and GIII. As it was commented

in regard to collagen, the fact that elastic fiber did not decrease after oxybutynin treatment may be explained by persistence of partial obstruction in these animals (group III) and the lack of a direct action of this drug on elastic fiber turnover.

There was a functional bladder improvement and the decrease of muscle bundle thickness in the GIII group after using intravesical oxybutynin, when compared with the group not treated with the drug. This could be related to an effect of oxybutynin on muscular growth, as it was inhibited in obstructed animals. Alternatively, this structural improvement may be associated with action of this drug on detrusor muscle [11].

Further studies are needed for a better understanding of the quantitative changes in the extracellular matrix during voiding dysfunction due to partial obstruction of bladder, as well as their regulatory mechanisms. The use of drugs that block this developing process may enable reversal or even interruption of the deterioration of the structural and functional properties of the bladder wall.

Conclusions

Intravesical oxybutynin seems to protect the bladder against functional and structural changes of OAB associated to PBOO. Additional studies will be important to confirm these data and to assess the effect of intravesical oxybutynin on bladder with low compliance and longer obstruction time.

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Conflicts of interest None.

References

1. Abrams P, Cardozo L, Fall M, Griffiths D, Rosier P, Ulmsten U et al (2002) The standardization of terminology of lower urinary tract function: report from the standardization sub-commitee of the International Continence Society. Neurourol Urodyn 21:167–178
2. Hyman M, Groutz A, Blaivas J (2001) Detrusitor instability in men: correlation of lower urinary tract symptoms with urodynamic findings. J Urol 166(2):550–552
3. Ewalt DH, Howard PS, Blyth B, Snyder HM 3rd, Duckett JW, Levin RM et al (1992) Is lamina propria matrix responsible for normal bladder compliance? J Urol 148:544–549
4. Cortivo R, Pagano F, Passerini G, Abatangelo G, Castellani I (1981) Elastin and collagen in the normal and obstructed urinary bladder. Br J Urol 53(2):134–137
5. Collado A, Batista E, Gelabert-Más A, Corominas JM, Arañó P, Villavicencio H (2006) Detrusor quantitative morphometry in obstructed males and controls. J Urol 176(6):2722–2728
6. Landau EH, Jayanthi VR, Churchill BM, Shapiro E, Gilmour RF, Khoury AE et al (1994) Loss of elasticity in dysfunctional bladders: urodynamic and histochemical correlation. J Urol 152:702–705
7. Lose G, Norgaard JP (2001) Intravesical oxybutynin for treating incontinence resulting from an overactive detrusor. BJU Int 87 (9):767–773
8. Yarker YE, Goa KL, Fitton A (1995) Oxybutynin: a review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic use in detrusor instability. Drugs Aging 6:243–262
9. Uvelius B, Mattiasson A (1984) Collagen content in the rat urinary bladder subjected to intravesical outflow obstruction. J Urol 132:587
10. Rubinstein M, Sampaio FJ, Costa WS (2007) Stereological study of collagen and elastic system in the detrusor muscle of bladders from controls and patients with infravesical obstruction. Int Braz J Urol 33(1):33–41
11. Yamamoto H, Kawano PR, Balasteghin KT, Padovani CR, Amaro JL (2009) Protective action of intravesical oxybutynin on bladder ultrastructure in rabbits with detrusor overactivity. Int Urogynecol J Pelvic Floor Dysfunct 20:229–234
12. Amaro JL, Balasteghin KT, Padovani CR, Montenegro R (2005) Structural alterations of the bladder induced by detrusor instability. Experimental study in rabbits. Int Braz J Urol 31(6):579–586
13. Balasteghin KT, Nardo AM, Amaro JL, Padovani CR (2003) Experimental model of bladder instability in rabbits. Int Braz J Urol 29:62–67
14. Junqueira LCU, Montes GS, Sanchez EM (1982) The influence of tissue section thickness on the study of collagen by the Picrosirius-polarization method. Histochemistry 74:153–156
15. Bancroft JD, Gamble M (2008) Theory and practice of histological techniques, 5th edn. Churchill Livingstone, Edinburgh, pp 1–725
16. Gundersen HJG, Bendtsen TF, Korbo L, Marcussen N, Moller A, Nielsen K et al (1988) Some new, simple and efficient stereological methods and their use in pathological research and diagnosis. APMIS 96:379–394
17. Cabral CAP, Sampaio FJB, Cardoso LEM (2003) Analysis of the modifications in the composition of bladder glycosaminoglycan and collagen as a consequence of changes in sex hormones associated with puberty or oophorectomy in female rats. J Urol 170:2512–2516
18. Bergman I, Loxley R (1963) Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. Anal Biochem 35:1961–1965
19. Zar JH (2007) Biostatistical analysis, 4th edn. Churchill Livingstone, Oxford, pp 1–744
20. Buyse G, Waldeck K, Verpoorten C, Bjork H, Casaer P, Andersson KE (1998) Intravesical oxybutynin for neurogenic bladder disfunction: less systemic side effects due to reduced first pass metabolism. J Urol 160:892–896
21. Matsumoto S, Chichester P, Kogan BA, Levin RM (2003) Structural and vascular response of normal and obstructed rabbit whole bladders to distension. Urology 62:1129–1133
22. Kim JC, Yoon JY, Seo SI, Hwang TK, Park YH (2000) Effects of partial bladder outlet obstruction and its relief on types I and III collagen and detrusor contractility in the rat. Neurourol Urodyn 19 (1):29–42
23. Inui E, Ochiai A, Nasa Y, Ukimura O, Kojima M (1999) Comparative morphometric study of bladder detrusor between patients with benign prostatic hyperplasia and controls. J Urol 161:827–830